Standards and Technologies for Early Cancer Biomarker Validation

Fluorophore probes for nucleic acids and antibodies, and high throughput, slide-based analyses are fundamental to many biomedical and clinical measurement systems. We have adapted a series of cancer biomarker DNA probes and antibodies for quantitative analysis in this format. High throughput is accomplished by using a programmable, robotically-actuated slide preparation. Analytes are tracked in cells and histopathology tissue sections by using secondary affinity detection fluorescence reagents that are subsequently quantitated by quantum dot fluorescence. With high throughput modifications, previously qualitative experiments such as fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) can be rendered quantitative and reproducible.

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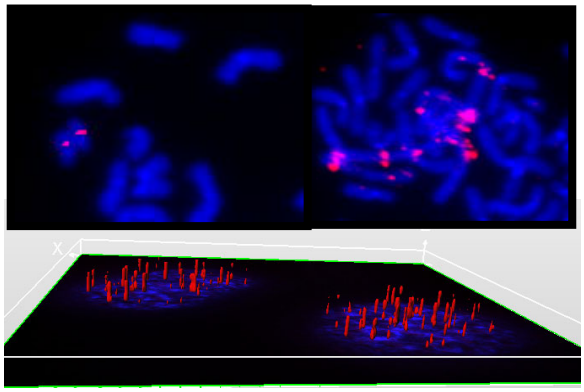
Quantum dots (semiconductor nanocrystals) offer advantages for bioimaging, especially as biological tags for clinical tests involving fluorescence microscopy.

NIST research has led to significant improvements of the reproducibility and assay performance of the HER2 biomarker that is needed to assure high-quality measurements for health care delivery.

Historically, biological imaging has traditionally relied upon radioisotopes and organic fluorophores for detection of proteins, RNA, and DNA sequences. However, the use of radioisotopes creates biohazard and disposal concerns. Because of this, organic fluorophores have been used for visual detection with fluorescence microscopy. However, photobleaching has compromised the quantitation and preservation of primary assay signals. The use of new inorganic fluorophores called quantum dots (semiconductor nanocrystals) has recently offered an alternative detection technology to organic fluorophores. NIST is partnering with industrial and other government laboratories to advance this technology.



Images of cancer analyte detection with quantum dots (red) superimposed on cellular genomic DNA (blue stain). Breast cancer biomarker HER2 DNA gene probe detected with qdot 605 fluorophore in metaphase chromosomes (blue objects). Left, normal gene copy signal (red). Right, amplified HER2 genes (red) in breast cancer cells. Lower panel: nuclear protein telomerase (red) quantitatively detected in high-expressing lung tumor cell nucleus (blue) with a NIST-designed IgY antibody probe.

Publications:

Xiao, Y., Barker P.E. **"Semiconductor nanocrystal probes for human metaphase chromosomes."** *Nucleic Acids Research* (2004) 32, e28.

Xiao, Y., Telford W.G., Ball, J.C., Locascio L.E., Barker, P.E. **"Semiconductor nanocrystal conjugates, FISH and pH."** *Nature Methods* (2005) 2, 723.